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TRANSFORMATION OF HYDROCARBONS BY MICROORGANISMS

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by Rebecca S. Bryant*

ABSTRACT

An increase in the number of microbial enhanced oil recovery projects in petroleum reservoirs has led to a heighted concern about the possible adverse effects that microbial metabolism may cause. One potential problem is the possibility of microbial metabolism on hydrocarbons in the reservoir, which might lead to undesirable alteration in the crude oil.

This topical report addresses the microbial activity on hydrocarbons, with an emphasis placed upon considering the possible environmental hazards that the application of microbial petroleum technology may cause.

From our analysis of existing data there is no proof that microorganisms have caused compositional changes in crude oil in petroleum reservoirs. There are a multitude of microorganisms that can metabolize crude oils aerobically, but there are no available anaerobic metabolic pathways for microorganisms to alter crude oils in the reservoir, where only anerobic conditions would exist. Certainly the advent of high technological processes could show that anaerobic degradation of petroleum by microorganisms can occur; however, at this time there is little chance of microbial anaerobic degradation occurring from microbial enhanced oil recovery processes.

INTRODUCTION

The chemical composition of crude oils and their insoluble character in water provide unusual substrates for microorganisms to degrade. Microorganism is a collective term used to describe organisms that must be observed under a microscope; the group includes bacteria, yeasts, protozoa, and fungi. Many members of these microbial taxa possess the capability to use hydrocarbons as their carbon and energy source of nutrient. During this use of hydrocarbon, they must be able to assimilate the compounds necessary for their continued metabolic activities, and their assimilation is made easier by the production of surface-active compounds. Bacterial species have a very rapid mutation

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rate (1 cell in 10^6), and they have an average generation time of 20 minutes. These properties allow microorganisms, particularly the single-celled bacteria and yeasts, to adapt to certain alterations in their environment. One type of adaptation that has been shown to occur is the increase in ability to break down or metabolize hydrocarbons after a prolonged exposure to the hydrocarbon compounds. Several investigators[1-4] have shown that if substrates such as crude oil or hydrocarbons are introduced into a marine or terrestrial environment, there will be a greater incidence of hydrocarbon-utilizing microorganisms, and a faster breakdown of hydrocarbons, than in areas where there is no substrate contamination.

Microorganisms are distributed ubiquitously in nature, and it has been postulated that even the heterogeneous microbial flora in aquatic and terrestrial environments includes naturally occurring hydrocarbon-degrading populations. Interestingly, Jensen[5] has reported that the amount of degradative adaption is limited by the concentration of hydrocarbon substrate that the microorganisms encounter. Jensen showed that if a higher concentration of hydrocarbon (greater than 5 percent) was added to an indigenous microbial population in soil, the microbial activity did not increase; however, at concentrations ranging from 0 to 5 percent hydrocarbon, the microbial population increased proportionally. Atlas[6] reported that the application of crude oil or diesel fuel on an Arctic coastal plain soil stimulated the microbial population over the next 7 years. It has been well documented that the crude oil spill from the Amoco Cadiz tanker was biodegraded to a great extent by marine microorganisms already present in the environment.[7]

It is beyond the scope of this review to consider all microbial transformations of hydrocarbons. This study reports on the tendencies of microorganisms to alter crude oil composition and provides guidelines for analyzing crude oils that have been altered and/or exposed to microbial activity. General biodegradation pathways will be briefly mentioned; however, biochemical metabolism of specific microorganisms will not be covered.

Microbial Biodegradation of Petroleum

Certain researchers have reported that microbial activity is one of the natural processes that changes the physical-chemical properties of crude oil in reservoirs.[8-9] An anonymous report has stated that approximately 10 percent of the world's crude oil has been destroyed by microbial activity.[10] Philippi[8] concluded that microbial populations have transformed billions of tons of primary paraffinic oils to medium-heavy gravity oils worldwide. An early study by Zobell[11] postulated that sulfate-reducing bacteria brought about the chemical changes in petroleum found in reservoirs. However, two later studies[12-13] were unable to support this hypothesis, and showed that the sulfate-reducing bacteria cannot contribute to the degradation of oil, although they can grow on residues caused by other microbial degradation. Cook and Westlake[13] studied interactions of sulfate-reducing bacteria (SRB) and aerobic petroleum-degrading bacteria. They found that neither pure nor mixed sulfate-reducers could degrade oils anaerobically. Sulfate-reducing bacteria could grow only on residues from aerobic bacterial degradation of the oils. Growth of SRB depended upon the aerobic species present, the oil type, and whether SRB were pure cultures or mixed. Oils tested were n-alkanes and crude oils.

Major crude oil type compositions are shown in table 1. Philippi[8] did an exhaustive study comparing crude oil compositions and classified crude oils as follows: (1) Paraffinic crude oils contain normal paraffins up to C-35. They are designated as primary because their chemical composition remains paraffinic and constant after expulsion from their source beds. (2) Heavy naphthenic crude oils contain very little gasoline and have a low paraffin content. (3) The light-to-medium gravity naphthenic crude oils (API 20° to 40°) are intermediate in composition between the primary paraffinic crude oils and the heavy naphthenics; most of this group contains some wax.

In general, crude oil contains 90 to 99 percent hydrocarbons; the remainder is made up of compounds containing sulfur, oxygen, and nitrogen, and trace amounts of metals. The relative concentrations of hydrocarbon and non-hydrocarbon compounds vary greatly, resulting in oils of widely differing properties. The major hydrocarbon components of crude oils are paraffins or alkanes (both straight and short branch chain compounds), followed by cycloalkanes (naphthenes), and smaller amounts of aromatic substances containing one or more benzene rings.

One of the best studied crude oils comes from Ponca City, Oklahoma.[14] This oil has been shown to contain at least 200 hydrocarbons that have been positively identified, including all of the n-paraffins (C_1-C_{32}); all four branched hexanes, six of eight branched heptanes, and 15 of 17 branched octanes; all possible C_8 isomers of alkyl cyclopentane and alkyl cyclohexane; and all 12 C_8 and C_9 isomers of alkyl benzene. In the higher boiling fractions, the large number of possible isomers with similar physical and chemical properties makes it unlikely that the composition of this crude oil can ever be fully resolved.

To study a crude oil, it is fractionated generally either by distillation or column adsorption chromatography. Table 2 illustrates a fraction of an Iranian crude oil, Agha Jari. The asphaltene (or heptane-insoluble) fraction has been defined only as having sheets of aromatic rings stacked on top of each other. The saturates contain normal and branched paraffins, cyclic paraffins, and alkyl cylic paraffins. The aromatic fractions contain large numbers of different classes of mono-, di-, and polyaromatic hydrocarbons and related sulfur-containing analogues. Each fraction can be further characterized by a combination of gas-liquid chromatography and mass spectrometry.

Parameters Influencing Petroleum Degradation

Biodegradation of alkanes and aromatic compounds is dependent upon a supply of molecular oxygen. One study[15] reports that the complete oxidation of 1 mg of hydrocarbon requires 3 to 4 mg of oxygen, and that the concentrations of dissolved oxygen found in surface waters (6 to 12 mg/l) are adequate to support microbial growth on thin oil slicks. An excellent example of a biodegraded oil slick occurring only at the surface was given by Blumer, et al.[16] After a severe fuel spill in Buzzard's Bay, Massachusetts, oil that had accumulated in the sediment was not degraded over several months, while near the surface of the sediment, significant degradation had taken place.

Several reports of anaerobic degradation of hydrocarbons can be found in the literature;[17] however, anaerobic degradation, if it occurs at all, does not contribute greatly to the ecology of petroleum biodegradation. McKenna and Kallio[18] postulated that anaerobic metabolism of n-alkanes is unlikely,

TABLE 1. - Composition of the most important crude oil types

Oil types	Gasoline: Content	Gasoline: Composition	Composition of 325° C residue
Paraffinic	High to low	Paraffinic	Waxy, n-paraffins high.
Light to medium naphthenic	High to low	More naphthenic, increased amount of branched-chain paraffins	Wax content normal to low.
Heavy naphthenic	Low to very low	Highly naphthenic, highly branched paraffins	n-paraffins very low to absent.

TABLE 2. - Fractionation of Agha Jari crude oil[1]

By distillation (30)				By column adsorption chromatography[2]	
Fraction	Boiling range, %	Vol, %	C atoms	Fraction	Vol, %
Gasoline	40-180	30.5	5-10	Asphaltene	1.7
Kerosene	180-230	9.5	10-13	Saturates	59.5
Gas-oil	230-300	14.5	12-17	Aromatics	14.0
Lubricant	300-400	17.5	17-26	Polar aromatics	23.2
Residue	>400	25	>24		

[1]See reference 14.
[2]A. Dieber, unpublished data.

because most microorganisms cannot grow on n-alkenes, the proposed intermediates for anaerobic metabolism of alkanes. If anaerobic biodegradation of petroleum does occur, the process cannot be a contributing factor to observed degradation of petroleum in nature. An interesting study by Schink[19] investigated the methanogenic bacteria to observe anaerobic biodegradation of hydrocarbons. He found that there was no stoichiometric degradation occurring with hexane, hexadecane, n-heptadecane, 1-hexene, cis-2-hexene, trans-2-hexene, isoprene, 1-hexine, benzene, toluene, xylene, cyclohexene, cycloheptatriene, cyclopentadiene, styrene, naphthalene, azulene, or beta-carotene. Squalene was incompletely converted to methane and carbon dioxide. Complete degradation occurred with hexadecane, and the mechanism was via beta-oxidation.

Temperature appears to be an important parameter for biodegradation of petroleum. Philippi[8] reports that in the five oil basins he studied, there was no oil transformation at subsurface temperatures exceeding approximately 150° F, while in three American basins, the cutoff temperature was 163° F. The maximum temperature tolerance of most microbial genera is also in these ranges. Since the rate of chemical reactions double approximately every 10° C, he could show that the temperature cutoff behavior of these oil basins was proof for microbial transformation as opposed to chemical or physical degradation. Stormer, et al.[20] used oil from the Ekofisk fields and the yeast, *Saccharomyces lipolytica*, to observe biodegradation. They varied the temperature from 8° to 20° C at an optimal pH of 8.5. The rate of biodegradation greatly increased at higher temperatures. A period of 3 to 16 days of incubation was used, and the degradation showed that normal alkanes with intermediate chain lengths were metabolized first, and branched ones last. The composition of the oil changed drastically, and the microbial culture also caused dispersion of the oil. After 16 days, all hydrocarbons up to phytane and pristane were gone.

Temperature may have some effects upon the specificity of microbial degradation of hydrocarbons. Jobson, et al.[3] showed that enrichment cultures from oil-contaminated soils at 4° or 30° C were equally effective in degrading North Cantal and Lost Horse Hill crude oils. The microbial population developed at 30° C used more compounds from the aromatic fraction than did the population at 4° C. Likewise, the microbial population at 4° C used more saturates from the crudes. Westlake, et al.[21] examined four crude oils at 4° and 30° C for biodegradation. Microbial populations that grew with an oil at 4° C metabolized a similar oil at 30° C, while populations that had grown at 30° C could not degrade oil readily at 4° C. Phytane and pristane were more resistant to biodegradation at 4° C than at 30° C.

Scarcely any work has been performed on the effects of pressure on biodegradation, because most biodegradation occurs at the surface level. Schwartz, et al.[22] used deep sea vent bacteria from a depth of 4,940 m to degrade n-hexadecane under 500 atm pressure at 20° C. They found that the rate at elevated pressure was much less than at 1 atm. A mixed culture of these deep sea bacteria degraded all components of a 19-component oil substrate.

In addition to temperature and oxygen concentrations, petroleum biodegradation is also influenced by several chemical parameters, including availability of nutrients for microbial metabolism. An early study by Zobell[11] showed that low concentrations of organic matter (less than 1 mg/1) promoted microbial action on hydrocarbons, probably by stimulating early growth of the organisms. McKenzie and Hughes[15] showed that higher concentrations of alternate carbon sources such as dextrose or fatty acids resulted in a decrease in the rate of oil oxidation. They concluded that under certain conditions, the fatty acid by-products of beta-oxidation on hydrocarbons may influence the rate of hydrocarbon degradation. The effect of nutrients upon petroleum biodegradation is still being investigated; however, it is general knowledge that the limitation of nitrogen and/or phosporous can present a problem for microorganisms in their attempt to utilize crude oil as their nutrient for carbon and energy. Atlas and Bartha[23] presented an interesting study regarding the supplement of nitrogen and phosphorous to crude oil biodegradation. They observed that only 3 percent of a Swedish crude oil was biodegraded and 1 percent transformed to CO_2 in freshly collected seawater. When nitrate or phosphate was added individually, there was little improvement in the extent of biodegradation. If both nutrient supplements were added together, they found that 70 percent of the crude oil was biodegraded and 42 percent was transformed to CO_2. Another related study showed that addition of nitrogen and phosphorous enhanced oil biodegradation in all 25 oligotrophic lakes studied in northern Wisconsin.[24]

Some trace minerals in the crude can have effects upon the biodegradation of the oil. Iron has a major impact on biodegradation rates. The concentration of iron that stimulates biodegradation was found by Dibble and Bartha[25] to be less than 1.2 mM; while the inhibitory concentration of iron was found to be approximately 5.2 mM.

In addition to chemical requirements for biodegradation of petroleum, certain abiological factors can influence this property. The rate and extent of biodegradation varies depending upon the type of crude oil present. Walker and Colwell[26] reported that in a comparison of two crude oils and two fuel oils, different populations of microorganisms were present. In that work, southern Louisiana crude oil with a low sulfur content was easily degraded, whereas Bunker C crude oil, which was high in sulfur and aromatic components, was not degraded readily.

The concentration of microorganisms at air-water interfaces can influence biodegradation rates of oils spilled in natural waters. Although some bacteria grow in the oil phase, bacteria capable of hydrocarbon oxidation generally grow at the oil-water interface, and their rate of growth is limited by the available interfacial area.[27] Dispersion of oil into small droplets greatly increases interfacial area, and thus the rate of microbial degradation. If a crude oil is exposed to a surfactant and wind and wave action, the oil will be removed at a faster rate. If a water-in-oil emulsion forms, then the biodegradation rate is very slow. Another dispersant study showed that some dispersants increased n-alkane loss from crude oils, whereas other dispersants had little effect.[28]

Specific Microbial Activities on Pure Hydrocarbons and Crude Oils

In most of the literature about microbial transformation of hydrocarbon compounds, the general parameters established for microbial specificity are as follows: (1) straight chain alkanes are degraded first; (2) branched and cyclic alkanes next; (3) then aromatics; (4) nitrogen and sulfur-containing compounds; and lastly, (5) asphaltenes. Other studies have shown that although the lower chain saturated components of crude oil disappear first, the specificity after that is not observed. Bailey[1] investigated a crude oil that was incubated for 21 days with four strains of aerobic bacteria. He found that the normal paraffins up to at least C_{34} were severely depleted, although the attack was temporarily blocked at C_{25}. The phytane and pristane components disappeared after the n-alkanes; and the lower ring naphthalenes and aromatics were attacked at the same time as the lighter normal paraffins. Interestingly, he found that additional non-hydrocarbon nitrogen and sulfur-containing compounds and asphaltenes were formed by the metabolism, and the oil was 30° API heavier than the initial reading.

Walker and Colwell[29] used a mixed saturated hydrocarbon substrate, which they termed a model petroleum, to evaluate the microbial population for hydrocarbon degradation in Chesapeake Bay water and sediment. Table 3 lists the components of their model system. Several techniques were used to determine the extent of degradation of this model oil. Oxygen consumption and biochemical oxygen demand indicated the rate of petroleum degradation, while dispersion and dry weight data determined the degree of emulsification of petroleum and total amount of petroleum degraded. The evolution of carbon dioxide and gas chromatography were used to identify the products of the degradation, as well as column chromatography of the model oil. The results of these studies were compared to identical experiments with motor oil. They observed an increased degradation of mono- and diaromatics using the model petroleum; however, the polyaromatics were degraded to a greater extent in the motor oil. Their studies show that a model petroleum comparison to crude oil degradation may be helpful for defining specific rates of degradation; however, at this time, not enough data are available for substantial conclusions.

Microbial Transformations of Alkanes

It is well established that microbial metabolism of alkane compounds exists, and that there is a vast and diverse population of microorganisms that can perform these degradative activities. Both short- and long-chain alkanes are oxidized at one terminal end to form the corresponding alcohol, aldehyde, and monobasic fatty acid.[30] The pathway for alkane metabolism is as follows: alkane ---→ alcohol ---→ aldehyde ---→ fatty acid ---→ acetate.[30] There is still some question as to the actual enzymology behind this degradative pathway. It is unclear whether microbial alkane oxidation is mediated by monooxygenases or dioxygenases. However, it is clearly established that

TABLE 3. - Composition of mixed saturated hydrocarbon substrate

Hydrocarbon	Percent
Normal alkanes:	
Decane	7.80
Undecane	7.80
Dodecane	7.80
Tridecane	7.80
Tetradecane	7.80
Pentadecane	7.80
Hexadecane	7.80
Heptadecane	7.80
Octadecane	7.80
Nonadecane	7.80
Eicosane	7.80
Branched alkanes:	
Pristane	3.90
Cyclic alkanes:	
Cyclohexane	3.90
Aromatics:	
Cumene	3.90
Naphthalene	0.66
Phenanthrene	0.66
Polynuclear aromatics:	
1, 2-Benzanthracene	0.39
Perylene	0.39
Pyrene	0.39

oxygen is a required reactant in the microbial oxidation systems for hydrocarbon degradation.

The metabolism of branched chain alkanes has primarily been evaluated by obtaining products of their degradation. Several microbial populations can degrade branched chain alkanes; and it appears that the 2-methyl branched alkanes are better substrates than are the 3-methyl branched alkanes.[14]

Alkane-degrading bacteria have several unusual visible morphological characteristics including intracellular cytoplasmic inclusions, and intracytoplasmic membranes. Numerous microbodies can occur in alkane-degrading yeasts.

Since long-chain alkanes are usually water insoluble, microorganisms must have a mechanism for uptake and transport of this alkane across its cell membrane. One mechanism is the emulsification of the alkane either into a macroemulsion or a microemulsion. Velankar, et al.[31] postulate that only the alkane present in micelles comes in contact with the bacterial cell surface. Zajic[32] has shown that biosurfactants also play a role in alkane uptake.

Microbial Activity on Cyclic Hydrocarbons

Alicyclic hydrocarbons are major components of crude oils. Table 4 presents an analysis of components in the gasoline fraction of several crude oils.[33] Alkyl-substituted cyclics are present in greater quantities than the parent cycloparaffin. Sachanen[34] showed that there are approximately five times more methylcyclopentane than cyclopentane, and methylcyclohexane is twice the amount of cyclohexane. Tar sands and shale oils also contain cycloparaffinic hydrocarbons. The literature does not contain as many references to the use of cyclic hydrocarbons by microbial populations as it does for the alkane compounds. When microorganisms that grow on n-paraffins have been isolated, they usually show negative results for cycloalkanes. The low solubility and their potential toxicity have been cited as possible reasons for the inability of microorganisms to grow on cyclic alkanes. Several microorganisms have been found to metabolize cyclic alkanes, including *Nocardia, Pseudomonas, Micrococcus,* and *Mycobacterium* species. In all cases, molecular oxygen is required for these degradative activities; thus, the potential of any microbial metabolism of cyclic alkane components in petroleum reservoirs is minimal.

TABLE 4. - The relative amount of paraffins, cycloparaffins and aromatics in the gasoline fraction of representative crude oils

Origin of the crude oil	Boiling range °C	Volume percent		
		Paraffins	Cyclo-paraffins	Aromatics
Oklahoma (Ponca City)	55-180	50	40	10
Pennsylvania	40-200	70	22	8
Texas (Hastings)	50-200	27	67	6
California (Santa Fe Springs	45-150	41	50	9
Canada (Turner Valley)	45-200	51	35	14
Mexico (Altamira)	40-200	49	36	14
Romania (Buscani)	50-150	56	32	12
Kuwait	40-200	72	20	8
Russia (Baku)	60-200	29	63	8

Microbial Activity on Aromatic Hydrocarbons

Considerable attention has been directed to the potential microbial degradation of aromatic hydrocarbons because of their widespread use and carcinogencity in the environment. Microorganisms can degrade aromatic hydrocarbons in both terrestrial and aquatic ecosystems.[14] The Eukaryotic and Prokaryotic microorganisms have the enzymatic capability to oxidize aromatic substrates that range in size from benzene to benzo(a)pyrene (5 aromatic rings). Species of microorganisms usually responsible for aromatic hydrocarbon degradation in nature include *Pseudomonas*, *Aeromonas*, *Moraxella*, *Beijerinckia*, *Flavobacterium*, *Achromobacter*, *Nocardia*, and *Corynebacterium*.[35] Many fungi are also capable of aromatic hydrocarbon biodegradation. The oxidation of these hydrocarbons initially begins with the incorporation of two atoms of molecular oxygen into the substrate to form a dihydrodiol with a cis-configuration.[30] This reaction is usually catalyzed by a dioxygenase enzyme. As with the biodegradation of alkanes and cyclic alkanes, oxygen is required for this reaction. Solanas and Pares[36] showed that a species of *Pseudomonas* could degrade Arabian light crude oil aerobically. The extent of alkyl naphthalene degradation was dependent upon the position, number, and type of substituents. After 20 days at 20° C, 40 to 50 percent of the oil residue was degraded. They isolated one strain that was more efficient at degrading aromatic hydrocarbons; it degraded 70 percent of the oil after 20 days incubation.

Microbial Transformation of Asphaltenes and Other Related Hydrocarbons

This group of compounds comprises the most difficult substrates for microbes to degrade. Walker and Colwell[26] showed that a mixed culture of bacteria could degrade southern Louisiana crude oil by decreasing the saturates and aromatics by 83.4 and 70.5 percent, respectively. The asphaltenes and resins increased by 28 percent, as did the C-28 through C-32 hydrocarbons. The order of degradation of aromatics was 6 rings, 1 ring, 2 rings, 3 rings, 5 rings, and then 4 rings, in decreasing order. Compounds containing sulfur were twice as difficult to degrade.

A very interesting study has been reported by Finnerty, et al.[37] They have isolated microorganisms that selectively oxidized specific sulfur and nitrogen-containing compounds. The bacteria were strictly aerobic and exhibited a specificity for either sulfur or nitrogen compounds. They were unable to grow with C_{10} to C_{20} alkanes, cycloalkanes, and mono- and polynuclear hydrocarbons. An increase of nutrient in the form of carbazole nitrogen enhanced the bacterial growth in the oil.

When Wyndham and Costerton[39] examined the degradation of bitumenous hydrocarbons, they observed colonization of tar sand coated filters. When they fractionated the oil, all fractions except the asphaltenes supported the growth. Again, the order of degradation was saturates, then aromatics, then the first polar fractions.

Microbial Degradation of Oil Shale

Westlake, et al.[38] compared the microbial degradation of raw and hydrogenated shale oil. They found that Gram-negative, rod-shaped bacteria predominated in all four oils used. The raw shale oil was resistant to microbial attack, whereas the hydrogenated shale oil was biodegradable. The hydrogenation process removes nitrogen- and sulfur- containing compounds. The n-alkanes still were degraded first, followed by the isoprenoids. They recommend that hydrogenation should take place at the site of extraction, prior to transport, in case there would be a spill.

Oil shales are general terms for a number of diverse fine-grained sedimentary rocks containing refractory organic material which can be retorted into fuels. Petrographic studies[40] indicate that organic materials are associated with clays, and the complete matrix is embedded in a cement of carbonate minerals. The entire system behaves as a composite with organics and inorganics tightly bonded. The permeability of oil shale is extremely low. The oil and gas yield of oil shale varies, but a general description includes bitumens which comprise 5 to 67 percent. These organic components are generally less than the inorganic constituents. The other type of organic is kerogen, which comprises the bulk of organic material in oil shale. While bitumen contains n-alkanes, branched and cyclic alkanes, aromatic oil, resins and asphaltenes, kerogen consists of polycyclic subunits interconnected by bridges of n-alkanes and isoprenoids. The matrix contains amounts of trapped alkanes, fatty acids, and bitumens.

Microorganisms are capable of undergoing metabolic activity on bitumen's alkanes, cyclics, and aromatics. However, there are resin and asphaltic fractions as well. *Pseudomonas, Mycobacterium,* and *Nocardia* have been shown to grow on asphalt systems. Kerogen has a very high molecular weight and is generally non-biodegradable by microorganisms.[40]

SUMMARY AND DISCUSSION

This report emphasizes the chemical heterogeneity and water insolubility of crude oil because these two factors make crude oils difficult to biodegrade. There are many petroleum-degrading microorganisms, probably due to the seepage of natural oil throughout centuries. The occurrence of man-made petroleum pollutants has probably only begun to cause an increase in the microbial populations that are capable of degrading petroleum hydrocarbons. At present, there appears to be no microbial system that can metabolize hydrocarbons anaerobically with any degree of efficiency. The potential certainly exists for some microbial systems to evolve mechanisms by which other components may serve as sources of molecular oxygen. The genus *Pseudomonas* comes close to this feat, being able to use nitrate without oxygen as a nutrient. The advance of genetic engineering certainly allows laboratory experimenters to genetically alter microorganisms so that they can more effectively degrade not only petroleum but also other chemical pollutants in the environment.

Oxygen concentration, temperature, pressure and nutrient availability are key parameters that influence microbial hydrocarbon degradation. The order of biodegradation of specific components in hydrocarbons appears to begin consistently with the straight chain alkane compounds. The branched and aromatic hydrocarbons are then degraded, and the asphaltene and nitrogen and sulfur-containing compounds are generally the last to be biodegraded.

It has been postulated that microorganisms have caused the chemical composition of oil to change, but to date, there is no concrete evidence that this has occurred in nature. Certainly aerobic biodegradation alters the chemical composition of petroleum, but no anaerobic biodegradation pathways in microorganisms have been proved.

The evolution of carefully controlled laboratory experiments and modern analytical techniques have enabled petroleum microbiologists to now investigate microbial transformation of hydrocarbons more competently, and it is assumed that in the next few years, the viewpoint of microbial transformation of hydrocarbons as only an aerobic process may be radically altered.

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